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IMMUNEFLUORESCENCE TEST IN THE DIAGNOSIS OF SYPHILIS

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(Polish Medical Weekly)
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The description of NELSON's test (TPI) opened a new direction in the serology of syphilis. This is characterized by the tendency to at least partially replace the hitherto used so-called classical tests for syphilis, which are based upon the use of non-specific lipoid antigens, with new methods which detect antibodies of syphilitic sera with a specific treponema antigen.

Following NELSON's discovery, several other treponema-pallidum (TP) tests were described, among others the TPIA (Treponema pallidum immunological adhesion), the TPA (Treponema pallidum agglutination), the TPMB (Treponema pallidum methylene blue staining), the TPCF (Treponema pallidum complement fixation with specific antigen). Although interesting theoretical observations could be made with these tests, none of them found wider practical employment in syphilis diagnostics because of their unsatisfactory replication, or the difficulty in the interpretation of their results.

NELSON's test is generally held, however, a decisive verificative method of the serological diagnosis of syphilis, and to a certain extent also a valued measure of the effectiveness of specific treatment. The procedure of this test requires a lot of work, and it costs much. Therefore, it cannot be used in the everyday diagnostic work, and particularly not for serological mass examinations.

In an effort at finding a simpler and cheaper method for the detection of specific antibodies in syphilitic sera, DEACON (2) undertook investigations into the use of immunefluorescence (IF) phenomena in syphilis diagnostics. In the test described by DEACON (2) ("Fluorescent Treponemal Antibody Test: FTA"), for which we propose the Polish name

"odczyn immunofluorescenji krętków (OIFK; immunefluorescence test for treponemas; here abbreviated as IFT or IFTT), the method of indirect IF was used as described by WELLER and COONS (9). This test runs in two phases. In phase one the treponema antigen, fixed on a slide, is bound with the antisyphilitic antibodies contained in the examined serum of the syphilitic patient. In the second phase of the test, from an immune serum which is prepared against human gamma-globulin and is labelled with fluoresceine isothiocyanate, a drop is added to the examined preparation. Then, an immunological bond is formed between the globulins of antitreponema antibodies and the labelled antiglobulin immune serum. Due to this double binding, the treponema antigen shows an intensive fluorescence. The result of the test is studied under a fluorescence microscope.

The essence of antibody detected in the IFTT hitherto has not been completely elucidated. Initially, it was identified with Nelsonian immobilized treponemas. Yet, soon it was experimentally proved (DEACON and HUNTER4; PILLOT and BOREL 8; COVERT et al., 1) that, with the aid of the IFT, in addition to antibodies specific for pathogenic Treponemata pallida, such antibodies are also detected which are against common, group protein antigens occurring in pathogenic as well as in saprophytic treponemas. The mentioned authors found that the antibodies active in the IFTT are different from the antibodies of the Wassermann test.

Further investigations were carried out on the scale of an international cooperation coordinated by the WHO (12; 13) whose purpose was to determine the sensitiveness, specificity, and replicability of these reactions. An agreement was reached that the sensitiveness of the IFT was superior to the sensitiveness of all other tests for syphilis. The antibodies detected by the IF method were manifested already in early syphilis, and then, earlier than in all others. In the opinion of FRIBOURG-BLANC and NIEL (3), the IFT becomes positive already at the moment of primary changes, and sometimes even earlier. On the basis of a large number of cases examined in the Białystok Laboratory, it was established that the IFTT's are manifest much earlier than the classical tests. The test is positive already on the first day after the manifestation of the primary lesion, and it reaches a rather specific high titre. In more than 90% of the patients with primary syphilis, the test was positive, and in 70% of them, the titre was more than 1:900, sometimes reaching rather high levels (MANIKOWSKA-LESIŃSKA, 6). In secondary and in early latent syphilis, the IFTT is always positive, and its quantitative titre reaches tens of thousands, many times exceeding the titre of the NELSON test. In the stage of late syphilis, a certain tendency is observed toward a reduction in the level of antibodies detected in the mentioned test. Observations of MANIKOWSKA-LESIŃSKA (6) and of FRIBOURG-BLANC and NIEL (5), hold, however, that even in this stage of syphilis the sensitiveness of the IFTT is not inferior to the sensitiveness of NELSON's test, and an independent self-negativization is a phenomenon almost as rare as in the NELSON test.

The specificity of the IFTT was the subject of numerous studies and lively discussions. In his first communication, DEACON (3) had already briefly stated that the percentage of non-specific results was rather large, if the test were carried out with non-diluted serum. Therefore, he proposed a 1:200 dilution of the examined serum. Just as the majority of authors using this method, DEACON (3) found likewise, that the specificity of this modification, known under the name of "FTA 200", was only very slightly inferior to the specificity of NELSON's test. Experiences in the Białystok Laboratory seem to prove that a 1:100 dilution also assures sufficient specificity for the reaction without loss of its high sensitiveness. Using diluted serum will essentially and greatly reduce the incidence of non-specific results in the test, but it does not eliminate them completely. For this reason, the IFTT cannot replace NELSON's reaction as a verificative method of the serological diagnosis of syphilis, although its specificity exceeds the specificity of classical syphilis tests.

The problem of replicability of the IFTT was the subject of joint researches carried out at the initiative of the WHO (12; 13). The ability of replicating the test conducted in different laboratories was established by comparison with the results obtained in DEACON's laboratory which was accepted as a model laboratory. The majority of laboratories were in concordance with DEACON's results within 90.5% to 97%. New observations of the authors who have a large experience at their disposal based upon many thousand tests indicate a perfect replicability of the IFTT, i.e., with both qualitative and quantitative method (FRIBOURG-BLANC and NIEL, 5; WAISMAN and HAMELIN, 10; MANIKOWSKA-LESIŃSKA, 6). From the experimental research of MANIKOWSKA-LESIŃSKA (6), it follows that the conditions of a good replicability of the test are careful preparation of the treponema antigen, and the possibly most accurate separation of treponemas from tissue remainder. To a still greater extent, the replicability of tests depends upon the use of high-quality labelled immune serum whose working dilution should be over 1:100.

Experiences of the Białystok Laboratory prove that the replicability of the quantitative IFTT is superior to all other quantitative tests used in the serology of syphilis. The large interval between titres facilitates accurate tracing of the variation in antibody level, which is extremely important in evaluating the results of specific therapy. In accordance with the experiences of FRIBOURG-BLANC (5), corroborated also by the research studies of MANIKOWSKA-LESIŃSKA (6), the quantitative IFTT facilitates to some extent the estimation of treponemal infective activity. The value of this test as a prognostic index, and particularly as a criterion for ruling out syphilis, requires further examinations.

Due to their extreme sensitiveness, high specificity, and rather low cost, the procedure of the IFTT tests can be useful in mass serological examinations. The simplified variant of the test is especially

suitable for area examinations (7; 11). The variant worked out by MANIKOWSKA-LESINSKA (7) is based upon experiences with the CHEDIAK test. The sensitiveness and specificity of this variant, consisting in performing the test with one drop of blood dried on a slide, was proved in numerous control examinations. Complete agreement was found between results of the simplified variant and of the original method. The simplified variant of the IFTT was tried out for mass serological examinations in the area of the Warsaw, Białystok, and Olsztyn voivodeships. Another simplified variant, being a continuation of DEMANCHE's blotting-paper technique, was worked out by WAISMAN, HAMELIN and GUTHE (11) with the idea to use the method in mass campaigns for the control of tropical treponematoses.

Research about the mechanism of the IFT, the improvement of its technique, and special elaboration of the principles for evaluating its results is still far from its completion. The hitherto acquired experiences show, however, that on the side of the NELSON test, the IFTT is a lasting and valuable enrichment of the modern methods for the sero-diagnosis of syphilis.

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